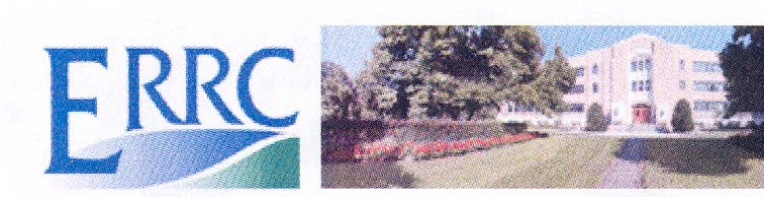

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Title: Radiation (Gamma) Resistance and Postirradiation Growth of *Listeria monocytogenes* Suspended in Beef Bologna Containing Sodium Diacetate and Potassium Lactate

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Radiation (Gamma) Resistance and Postirradiation Growth of *Listeria monocytogenes* Suspended in Beef Bologna Containing Sodium Diacetate and Potassium Lactate†

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ABSTRACT

Listeria monocytogenes, a psychrotrophic foodborne pathogen, is a frequent postprocessing contaminant of ready-to-eat (RTE) meat products, including frankfurters and bologna. Ionizing radiation can eliminate *L. monocytogenes* from RTE meats. When they are incorporated into fine-emulsion sausages, sodium diacetate (SDA) and potassium lactate (PL) mixtures inhibit the growth of *L. monocytogenes.* The radiation resistance of *L. monocytogenes,* and its ability to proliferate during long-term refrigerated storage (9°C), when inoculated into beef bologna that contained 0% SDA–0% PL, 0.07% SDA–1% PL, and 0.15% SDA–2% PL, were determined. The radiation doses required to eliminate 90% of the viable *L. monocytogenes* cells were 0.56 kGy for bologna containing 0% SDA–0% PL, 0.53 kGy for bologna containing 0.07% SDA–1% PL, and 0.46 kGy for bologna containing 0.15% SDA–2% PL. *L. monocytogenes* was able to proliferate on bologna containing 0% SDA–0% PL during refrigerated storage, but the onset of proliferation was delayed by the addition of the SDA-PL mixtures. An ionizing radiation dose of 3.0 kGy prevented the proliferation of *L. monocytogenes* and background microflora in bologna containing 0.07% SDA–1% PL and in bologna containing 0.15% SDA–2% PL over 8 weeks of storage at 9°C. Little effect on lipid oxidation and color of the control bologna, or bologna containing SDA-PL mixtures, was observed upon irradiation at either 1.5 or 3.0 kGy.

Listeria monocytogenes is a frequent postprocessing contaminant of ready-to-eat (RTE) meat products and has been associated with a number of foodborne illness outbreaks *(4, 5, 13, 25).* It is capable of growth at refrigerated temperatures and in high-salt environments, and thus it can proliferate during long-term storage *(17).* Because of the high mortality rate associated with *L. monocytogenes* infection, the pathogen is given zero tolerance in RTE meat products in the United States *(9, 23).*

Ionizing radiation (irradiation) is effective in the elimination of *L. monocytogenes* from RTE meats *(6, 18–21).* In previous work it has been determined that a radiation dose of 2.5 to 3.7 kGy is required to eliminate 5 log units of *L. monocytogenes* from vacuum-packaged RTE meats, depending on the product formulation *(6, 21).* A petition to allow ionizing radiation pasteurization of RTE foods, including RTE meat products such as bologna and frankfurters, has been filed with the U.S. Food and Drug Administration *(12).*

Sodium diacetate (SDA) and potassium lactate (PL) and combinations of the two can inhibit the growth of *L. monocytogenes* on RTE meats during long-term refrigerated storage *(3, 8, 14–16, 20, 22).* A combination of the two compounds is more effective than either compound alone. Although the two compounds are bacteriostatic against *L. monocytogenes,* they are not bacteriocidal against the microorganism when they are incorporated into the meat emulsion *(3, 15, 20).* While SDA and PL are approved for use in RTE meat products *(24),* few if any data on the effect of ionizing radiation in combination with lactate-diacetate mixtures on the viability and growth potential of *L. monocytogenes* exist.

In this study, the following questions were addressed. (i) What are the effects of ionizing radiation and SDA-PL mixtures on the radiation resistance of *L. monocytogenes* inoculated into cooked beef bologna emulsion? (ii) What are the effects of ionizing radiation and SDA-PL mixtures on the postirradiation growth of *L. monocytogenes*? (iii) What are the effects of SDA-PL mixtures and ionizing radiation on bologna lipid oxidation and color? To the knowledge of the authors, this is the first report on the interaction between ionizing radiation and SDA-PL mixtures on the radiation resistance and postirradiation growth of *L. monocytogenes.*

MATERIALS AND METHODS

Sausage manufacture. Ground beef (15% fat) was mixed in a Hobart Model HCM40 Cutter-Mixer. Cure ingredients and additives (wt/wt per kg of meat) included 3% sodium chloride, 3% dextrose, 0.5% sodium tripolyphosphate, 0.05% sodium erythorbate, 0.02% sodium nitrite, and 30% deionized water. The SDA-PL mixture (Purasol P Optiform-4, Purac-America, Lincolnshire, Ill.) was added as needed to obtain the required final concentrations and represented the only experimental variable other than

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† Mention of a brand or firm name does not constitute an endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

radiation dose. To limit the number of experimental variables, spices were not used. The emulsion mixture, without (control) or with SDA-PL mixtures (0.07% SDA–1% PL or 0.15% SDA–2% PL), was stuffed into 10-cm fibrous casings (Dewied Int., Santa Fe, N.M.). The sausages were then cooked in a Koch Model KL-50 Smokehouse (Koch Inc., Kansas City, Mo.) to an internal product temperature of 73°C. The dry bulb setting was 90°C, and the wet bulb setting was 63°C, for a relative humidity of ca. 47%.

After the appropriate internal temperature was reached, the sausages were immediately chilled in a sterile cold-water bath, and their casings were removed in an aseptic manner. The sausages were then sectioned and vacuum packaged to 0.5 mm Hg (ca. 66.7 Pa) with a Multi-Vac A300 Vacuum Packager (Multi-Vac, Kansas City, Mo.), overpacked in gas- and moisture-impermeable Mil-B-131-H foil bags (Bell Fibre Products Corp., Columbus, Ga.), and stored at 0 to 2°C until they were used. The pHs of bolognas containing no SDA-PL, 0.07% SDA–1% PL, and 0.15% SDA–2% PL were 6.8, 6.5, and 6.2, respectively.

Strains. Four *L. monocytogenes* strains (H7762 serotype 4b, H7764 serotype 4b, F4249 serotype 1/2a, and F4561 serotype 1/2a) were obtained from the Centers for Disease Control and Prevention (Atlanta, Ga.). The strains were propagated on Palcam agar (Difco Laboratories, Detroit, Mich.) at 37°C and maintained at 0 to 2°C until they were used. The identity of *Listeria* was confirmed by Gram staining followed by analysis with gram-positive identification cards with the Vitek Automicrobic System (bioMerieux Vitek, Inc., Hazelwood, Mo.).

***D_{10}*-values and extended storage.** Each *L. monocytogenes* strain was cultured independently in 100 ml of tryptic soy broth (Difco Laboratories, Sparks, Md.) in baffled 500-ml Erlenmeyer culture flasks at 37°C at 150 rpm for 18 h. The cultures were then combined and centrifuged at 1,725 × *g* for 30 min. The *L. monocytogenes* mixture was then resuspended in 40 ml of Butterfield's phosphate buffer (BPB; Applied Research Institute, Newtown, Conn.), inoculated into the loosely emacerated cooked bologna emulsion to a density of ca. 1×10^8 CFU/g, mixed by stomaching for 90 s, and 5-g aliquots transferred to no. 400 stomacher bags. The stomacher bags were then vacuum packaged at 0.5 mm Hg (ca. 66.7 Pa), overpacked in gas-impermeable Mil-B foil bags (Bell Fibre Products), and vacuum packaged again with a Multi-Vac A300 packager. Samples were refrigerated at 0 to 4°C for ca. 60 min prior to irradiation.

The same inoculation procedure was used for the extended-storage studies with the exception that the microorganism levels were adjusted so that $\leq 10^3$ CFU/g would be obtained following the application of ionizing radiation at 0-, 1.5-, and 3.0-kGy doses. Following irradiation, the samples were stored at 9°C for 8 weeks to simulate mild temperature abuse.

Following irradiation, the samples were assayed for cell counts by standard pour plate procedures. Forty-five milliliters of sterile BPB was added to a no. 400 stomacher bag that contained a 5-g inoculated sample, and the sample was mixed by stomaching for 90 s. The samples were then serially diluted in BPB using 10-fold dilutions, and 1 ml of diluted sample was pour plated using *Listeria* specific Palcam agar or tryptic soy agar (TSA) (Difco). Palcam agar was used to determine the radiation doses required to eliminate 90% of the viable *L. monocytogenes* cells (D_{10}-values). The nonspecific TSA medium was used in addition to Palcam agar during the extended-storage study to quantify the background microflora. Three 1-ml aliquots per dilution were plated. The plates were then incubated for 48 h at 37°C prior to enumeration of colonies. The average count (CFU/g) for an irradiated sample (N) was divided by the average count (CFU/g) for the untreated control (N_0) to produce a survivor ratio (N/N_0). D_{10} was determined by calculating the reciprocal of the slope provided by the $\log(N/N_0)$ ratios versus irradiation dose *(21)*.

Gamma irradiation. A self-contained ^{137}Cs radiation source (Lockheed Georgia Company, Marietta, Ga.) was used for all exposures. The radiation source consisted of 23 individually sealed source pencils placed in an annular array. The cylindrical sample chamber (22.9 by 63.5 cm) was located central to the array when placed in the operating position. The dose rate, which was verified according to ASTM method E 2116-00 *(2)* with dosimeters obtained from the National Institute of Standards and Technology, was 0.096 kGy/min. The temperature during irradiation was maintained at 4.0 ± 1.0°C by the gas phase of a liquid nitrogen source that was introduced directly into the top of the sample chamber. To ensure that a uniform radiation dose was delivered, bags were placed centrally and vertically within the cylindrical chamber. The temperature was monitored with two thermocouples placed on the sides of the sample bags. The dose delivered was verified with 5-mm alanine pellet dosimeters that were attached to the sides of the sample bags, which were then measured with a Brucker EMS 104 EPR Analyzer (Brucker, Billerica, Mass.). Recorded doses were typically within 5% of the target doses. The radiation doses used for D_{10} determination were 0, 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 kGy. Radiation doses of 0, 1.5, and 3.0 kGy were used for growth promotion and quality factor experiments.

Lipid oxidation. Lipid oxidation was measured by the thiobarbituric acid (TBA) assay modified from the methods of Hodges et al. *(7)* and Zipser and Watts *(26)*. Ten grams of bologna was homogenized with 20 ml of 0.5 M phosphate (pH 2.5) buffer containing 0.08% sulfanilamide and 0.01% butylated hydroxytoluene with the use of a homogenizer (Virtishear, Virtis, Gardiner, N.Y.) at a speed setting of 70 for 1 min. The homogenate was centrifuged at 6,700 × *g* for 10 min at 5°C in a Sorvall R2C-B refrigerated centrifuge (Kendro Laboratory Products, Newtown, Conn.) and was then filtered through a Whatman no. 11 paper filter (Whatman, Inc., Clifton, N.J.). The filtrate was centrifuged at 1,300 × *g* for 10 min at 23°C in a Sorvall RT6000B refrigerated vcentrifuge (DuPont Co., Wilmington, Del.). A 1.6-ml aliquot of the supernatant was added to a test tube containing 1.6 ml of either −TBA solution (15% [wt/vol] trichloroacetic acid and 0.01% butylated hydroxytoluene) or +TBA solution (15% [wt/vol] trichloroacetic acid, 0.01% butylated hydroxytoluene, and 0.65% TBA). Samples were then mixed vigorously, heated at 95°C in a water bath for 25 min, cooled, and centrifuged at 1,300 × *g* for 10 min at 5°C. Absorbance levels at 440, 532, and 600 nm were measured with a Shimadzu UV-1601 spectrophotometer (Shimadzu Scientific Instruments, Columbia, Md.). TBA reactive substance values were expressed as malondialdehyde (MDA) equivalents and calculated by the formulas developed by Hodges et al. *(7)*:

$$[(\mathrm{Abs532}_{+\mathrm{TBA}} - \mathrm{Abs600}_{+\mathrm{TBA}}) - (\mathrm{Abs532}_{-\mathrm{TBA}} - \mathrm{Abs600}_{-\mathrm{TBA}})] = A \quad (1)$$

$$[(\mathrm{Abs440}_{+\mathrm{TBA}} - \mathrm{Abs600}_{+\mathrm{TBA}})0.0571] = B \quad (2)$$

$$\mathrm{MDA}\ (\mathrm{nmol \cdot g^{-1}}) = [(A - B)/157{,}000]10^6 \quad (3)$$

Color analysis. Sausage pieces were packed and irradiated as described previously. Color analysis was then carried out with the use of a Hunter Lab Miniscan XE Meter (Hunter Laboratory, Inc., Reston, Va.) *(10, 11)*. The meter was calibrated with white and black standard tiles. Illuminate D65, a 10° standard observer, and a 2.5-cm port–viewing area were used. Six readings were taken for each parameter.

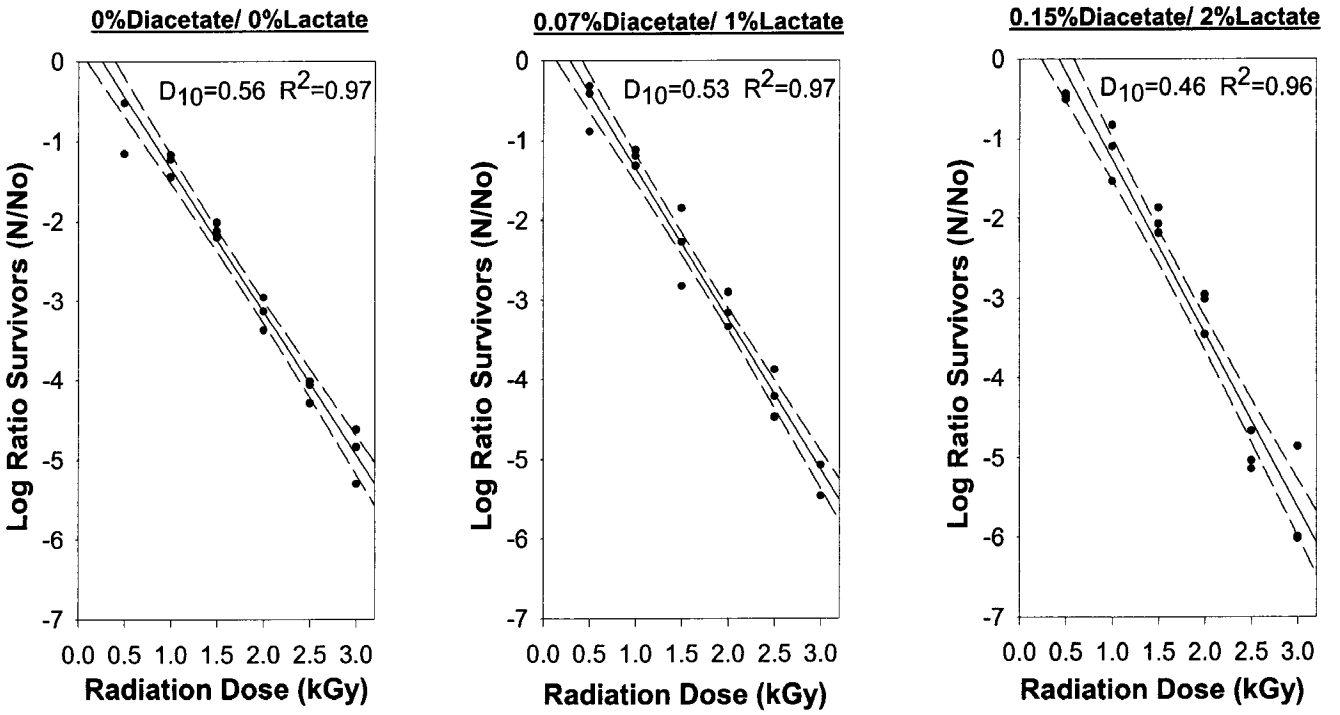

FIGURE 1. *Radiation resistance of* L. monocytogenes *suspended in cooked beef bologna containing various levels of SDA and PL. The linear regression is shown as a solid line, and 95% confidence intervals are shown as dashed lines. Individual log reduction values are shown as closed circles. Each experiment was conducted independently three times.*

RESULTS

Bologna containing 0% SDA–0% PL, 0.07% SDA–1% PL, or 0.15% SDA–2% PL was inoculated with *L. monocytogenes* at ca. 10^8 CFU/g. No effect of the SDA-PL mixtures on the viability of *L. monocytogenes* was observed during the 60-min refrigerated-storage period prior to irradiation. D_{10}-values (Fig. 1) for *L. monocytogenes* inoculated onto bologna containing 0% SDA–0% PL (control), 0.07% SDA–1% PL (low concentration), and 0.15% SDA–2% PL (high concentration) were 0.56 ± 0.02, 0.53 ± 0.03, and 0.46 ± 0.02 kGy, respectively. The radiation resistance of *L. monocytogenes* inoculated onto high-concentration SDA-PL bologna was significantly (18%) lower than that of *L. monocytogenes* inoculated onto control bologna as determined by analysis of covariance ($n = 3$, $\alpha = 0.01$).

The ability of *L. monocytogenes* to proliferate during long-term refrigerated storage was evaluated. The effect of mild temperature abuse at 9°C was investigated in this study. *L. monocytogenes* and background microflora on control bologna were able to proliferate easily during the 8-week storage period (Fig. 2). *L. monocytogenes* on control bologna irradiated to 1.5 kGy was able to recover quickly and reach a density of 6 to 8 log CFU/g within 4 weeks. Although growth was erratic, *L. monocytogenes* and background microflora reached a density of 4.5 log CFU/g (from an initial density of 2 log CFU/g) in control bologna irradiated to 3.0 kGy by the end of the 8-week storage period (Fig. 2).

L. monocytogenes was able to proliferate easily in nonirradiated low-concentration SDA-PL bologna (Fig. 2). Irradiation at 1.5 kGy was able to slow *L. monocytogenes* proliferation, while irradiation at 3.0 kGy completely inhibited the growth of both *L. monocytogenes* and the background microflora in low-concentration SDA-PL bologna. *L. monocytogenes* was unable to proliferate in high-concentration SDA-PL bologna regardless of the radiation dose. An ionizing radiation dose of 1.5 or 3.0 kGy was able to inhibit the growth of background microflora in addition to that of *L. monocytogenes* (Fig. 2).

Irradiation and SDA-PL mixtures had little effect on lipid oxidation of the bologna (Table 1). Compared with control samples, samples containing SDA-PL mixtures were lighter in color (with higher L values) regardless of the radiation dose (Table 2). Regardless of the radiation dose, the addition of 0.15% SDA–2% PL increased a values (redness) compared with those of the control samples. Irradiation had little effect on a values. SDA and PL did not have any effect on b values (yellowness). Irradiation increased b values, but only for samples that did not contain SDA and PL (Table 2).

DISCUSSION

Ionizing radiation can eliminate *L. monocytogenes* from fine-emulsion sausage such as frankfurters or bologna *(6, 18–21)*. Sommers and Thayer *(21)* found that the ability of ionizing radiation to eliminate *L. monocytogenes* was dependent on product formulation, with doses of 2.5 to 3.7 kGy being needed to provide a 5-log reduction. In other work, Foong et al. *(6)* found that ionizing radiation doses of 2.5 to 3.0 kGy were needed to eliminate 5 log units of *L. monocytogenes* inoculated onto four different types of RTE meats. Al-Bachir and Mehio *(1)* found that ionizing radiation extended the shelf lives of various luncheon meats by ca. 2 weeks without significant effects on product quality.

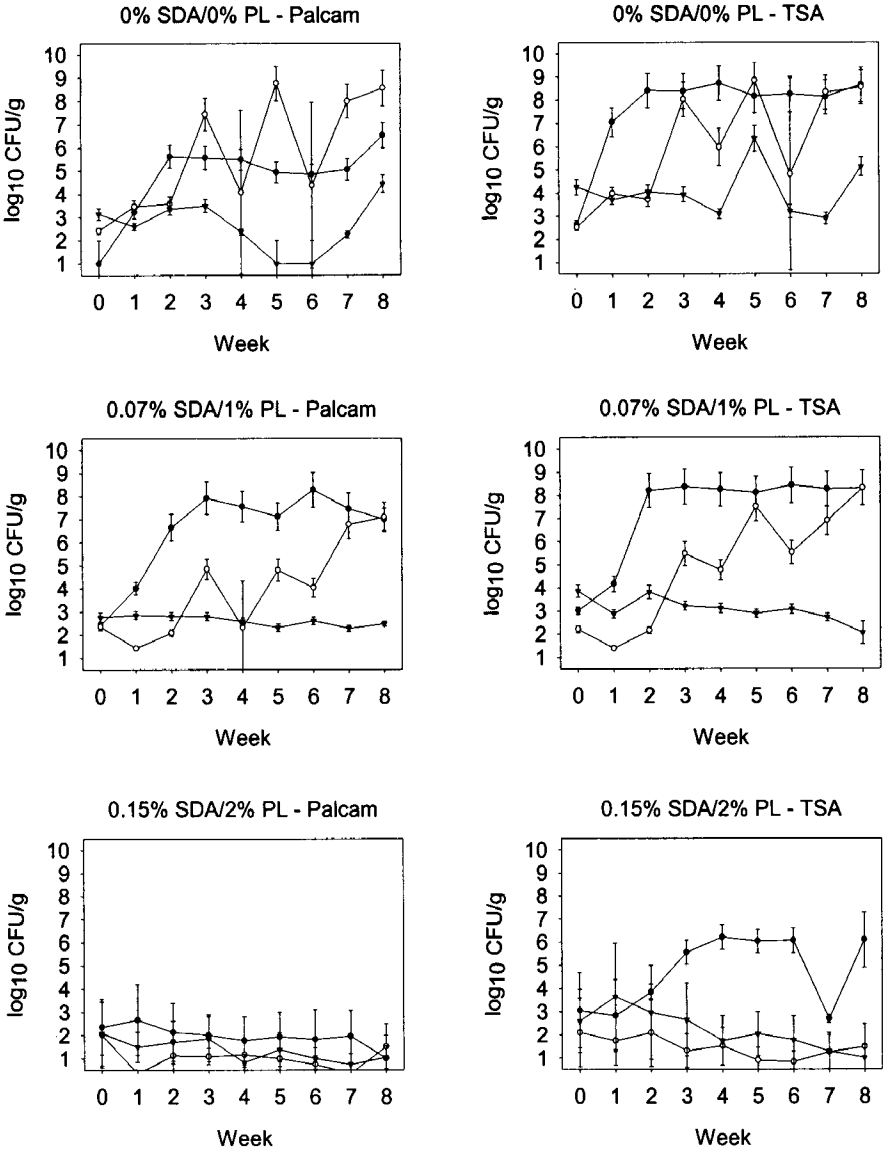

FIGURE 2. *Proliferation of* L. monocytogenes *suspended in cooked beef bologna containing various levels of SDA and PL over an 8-week period of refrigerated (9°C) storage. Unirradiated samples are represented by closed circles, samples irradiated to 1.5 kGy are represented by open circles, and samples irradiated to 3.0 kGy are represented by triangles. Each experiment was conducted independently three times. Standard errors of the means are shown as bars for each point.*

The radiation resistance of *L. monocytogenes* can be affected by the use of ingredients in the meat emulsion or by the application of surface treatments prior to packaging. Incorporation of the extender and antioxidant soy protein concentrate increased the radiation resistance of *L. monocytogenes* such that a dose of 3.7 kGy was needed to eliminate 5 log units of the microorganism *(19)*. Surface treatments including the application of citric acid were found to decrease the radiation resistance of *L. monocytogenes* that had been surface inoculated onto frankfurters and to inhibit *L. monocytogenes* postirradiation proliferation during refrigerated storage *(18)*.

TABLE 1. *Effects of ionizing radiation and sodium diacetate (SDA)–potassium lactate (PL) mixtures on lipid oxidation of beef bologna*[a]

Treatment	TBARS value for radiation dose			LSD
	0 kGy	1.5 kGy	3.0 kGy	(0.05)
0% SDA–0% PL	11.97	12.97	13.06	2.26
0.07% SDA–1% PL	11.89	12.79	13.27	0.88
0.15% SDA–2% PL	12.28	11.66	12.24	1.20
LSD (0.05)	2.03	1.27	1.26	

[a] The effects of ionizing radiation and SDA-PL mixtures on beef bologna lipid oxidation were determined by the thiobarbituric reactive substances (TBARS) assay. Statistical significance was determined on the basis of the least square difference (LSD) ($n = 3$, $\alpha = 0.05$).

Additives that can prevent the proliferation of *L. monocytogenes* in RTE meats include SDA, PL, and mixtures of the two. Both compounds have been approved for use in RTE meats *(24)*. The ability of these compounds to inhibit the growth of, but not eliminate, *L. monocytogenes* during long-term refrigerated storage has been well established *(4, 8, 14–16, 22)*. Recent work has indicated that SDA could increase the radiation sensitivity of *L. monocytogenes* at concentrations of ≥0.5% when applied to product surfaces or incorporated into emulsions *(20)*. Unfortunately, the 0.5% SDA concentration exceeds the limit allowed by current U.S. regulations *(24)*. Lower concentrations of SDA used in combination with ionizing radiation have been found to slow, but not prevent, the proliferation of *L. monocytogenes* surviving irradiation *(20)*. Lactate can

TABLE 2. *Effects of ionizing radiation and sodium diacetate (SDA)–potassium lactate (PL) mixtures on the color of beef bologna*[a]

Treatment	0 kGy	1.5 kGy	3.0 kGy	LSD (0.05)
		L values		
0% SDA–0% PL	49.5	48.2 B	48.7	0.8
0.07% SDA–1% PL	51.4 A	49.6 A	49.7 A	1.0
0.15% SDA–2% PL	52.0 A	50.5 AB	51.3 A	0.9
LSD (0.05)	1.2	0.7	0.7	
		a values		
0% SDA–0% PL	12.4	12.9	13.0	0.7
0.07% SDA–1% PL	12.7	13.6	13.2	1.1
0.15% SDA–2% PL	13.6 A	15.5 AB	14.2 A	0.8
LSD (0.05)	0.7	0.9	1.0	
		b values		
0% SDA–0% PL	14.2	15.0 B	14.8 B	0.5
0.07% SDA–1% PL	14.0	14.7	14.1	0.8
0.15% SDA–2% PL	13.5	14.9 B	14.0	0.8
LSD (0.05)	0.8	0.6	0.8	

[a] Statistical significance was determined on the basis of least square difference (LSD) ($n = 6$, $\alpha = 0.05$). Statistical significance by radiation dose (A) and by SDA-PL concentration (B) is shown for each value.

inhibit the growth of *L. monocytogenes* on frankfurters, but it does not increase the radiation sensitivity of the microorganism (C.S., unpublished data).

In this study, the ability of SDA-PL mixtures in combination with ionizing radiation to eliminate *L. monocytogenes* and inhibit its postirradiation proliferation was evaluated. Ionizing radiation and SDA-PL mixtures can delay the proliferation of *L. monocytogenes,* as shown in Figure 2. The radiation resistance of *L. monocytogenes* decreased by 18% when the pathogen was inoculated into beef bologna emulsion containing 0.15% SDA–2% PL. This SDA-PL mixture then inhibited the postirradiation growth of *L. monocytogenes* that survived irradiation for a period of 8 weeks at an abuse temperature of 9°C. Concentrations of both compounds were within current regulatory limits. While the lower SDA-PL concentration did not affect the initial radiation resistance of *L. monocytogenes,* the negative impact on the ability of *L. monocytogenes* cells that survived irradiation (3.0 kGy) to proliferate demonstrated the advantage of using this listeriostatic mixture in RTE meats. These data indicate the potential use of ionizing radiation in combination with SDA-PL mixtures in preventing listeriosis due to postprocessing contamination of fine-emulsion sausages or in preventing *L. monocytogenes* growth in accidentally recontaminated packages of irradiated RTE meats. The addition of SDA-PL mixtures did not negatively impact the color or the lipid oxidation of beef bologna.

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